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A genome-wide investigation of autozygosity and breast cancer risk

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14. ABSTRACT Long segments (> 1 megabase) of homozygous DNA are common in the genomes of outbred human populations. Several lines of research indicate that elevated genome-wide homozygosity and "runs of homozygosity" (RoH) at specific loci may increase breast cancer risk. In this project, we determine if genome-wide RoH content and individual RoHs are more common in breast cancer cases than in controls, using logistic regression methods. Using genome-wide SNP data (525,000 SNPs) on 1,647 non-Hispanic white, early-onset premenopausal breast cancer cases and 1,556 matched controls we identified over 65,000 individual RoHs and 423 genomic regions harbor RoHs for at least 10 individuals in our dataset (i.e., "RoH regions"). Overall RoH content was not associated with breast cancer status or with subtypes of breast cancer as defined by estrogen receptor status. Furthermore, analyses of each of the 423 RoH regions did not reveal any region in which RoH status was significantly associated with breast cancer risk or risk for a breast cancer subtype (after correction for multiple testing). Finally, comparing the RoH regions showing the strongest associations in our study to the regions with the strongest association in a prior study of RoHs in breast cancer did not reveal any common findings across studies. In this association study of RoHs and early-onset breast cancer risk, we have not implicated overall RoH content or any individual RoH in breast cancer risk. We are currently pursuing sequencing of candidate genes believed to be important for risk for ER-negative breast cancer.					
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INTRODUCTION

Long segments (> 1 megabase) of homozygous DNA are common in the genomes of outbred human populations (1-5). Several lines of research indicate that elevated genome-wide homozygosity (i.e. autozygosity) may increase breast cancer risk (6-9). For several cancer types, small studies have found increased germline homozygosity at specific genomic locations, suggesting these regions harbor important cancer genes (10, 11). Homozygosity mapping is a natural extension of large genome-wide association studies and has the potential to identify novel breast cancer genes and provide biological insights. Based on this evidence, we hypothesize that germline autozygosity is more common in breast cancer cases than in controls. More specifically, we hypothesize that there are specific regions of the genome in which homozygosity (i.e. "runs of homozygosity" (RoHs)) are more common in breast cancer cases than in controls and that these regions contain breast cancer-related genes.

BODY

Description of progress towards accomplishing tasks in scope of work:

Task 1 data acquisition and preparation: We have obtained all the genotype data for all 3,203 participants included in this project. We have performed all standard quality control procedures for all participants, including removal of population outliers and samples of poor quality; SNP filtering based on call rates, Hardy-Weinberg Equilibrium, and minor allele frequency; and assessment of and adjustment for population structure.

Task 2: genome-wide autozygosity analysis: We have identified runs of homozygosity (RoH) of at least 1 megabase in our data using two different methods, as implemented in the Golden Helix and PLINK programs, respectively. For both methods, >66,000 RoHs were detected. We used these RoHs to derive genome-wide measures of overall homozygosity. We have tested these measures for association with case/control status and also performed sub-group analyses by estrogen receptor status. No significant association between number or length of RoH segments and breast cancer risk, including analyses stratified by estrogen receptor status

Task 3: Conduct autozygosity mapping analysis: using the Golden Helix data on genome-wide RoHs, we have identified 423 regions in which homozygosity is somewhat common (>10 occurrences in our dataset). However, we did not observe any significant association between any specific RoH segment and breast cancer risk. In analyses stratified by estrogen receptor status, no significant associations were observed.

Task 4: CGEMS analysis: RoH analysis in the publically available CGEMS dataset of primarily ER-positive breast cancer has recently been published by another group (12), and we have compared our findings to this work. While significant associations were observed in neither study, we found no overlap between our most significant RoH regions and the regions identified in the CGEMS analysis.

Task 5: DNA Sequencing: Because our RoH results did not provide interesting regions for sequencing, we have sequenced several regions known to be important in ER- breast cancer including 19p13 (several genes), ESR1, RAD51L1, and TOX3, in order to discover new variants in these regions and assess evidence of association for low frequency variants. The sequencing of these genes is now complete, and genotype calls have been generated for approximately 190 early-onset ER- cases and 190 age-matched controls. Data is currently being analyzed using the PLINK/SEQ software to conduct gene-level association tests using information on rare coding variants.

KEY RESEARCH ACCOMPLISHMENTS

- Obtaining and performing quality control procedures on GWAS data
- Estimation and description of RoHs estimated from GWAS data.
- Test of runs of homozygosity (both genome-wide RoH levels and individual loci) for association with early-onset breast cancer
- Assessment of associations between RoHs with breast cancer subtypes
- Assessment of associations for SNPs at known breast cancer susceptibility loci with risk breast cancer subtypes among early onset cases
- Selection of genes for sequencing based on subtype specific associations
- Sequencing and genotype calling for ~20 candidate genes among ~190 ER- breast cancer cases and ~190 age-matched controls

REPORTABLE OUTCOMES

- This work was presented at the Era of Hope meeting to be held in August 2011 in Orlando, FL.
- This Award has supported the post-doctoral training that helped the P.I. receive several job offers for tenure-track faculty positions.
- We are working on a publication that will report on our results for the sequencing of ~20 candidate genes among ~190 ER- breast cancer cases and ~190 age-matched controls.

CONCLUSION

In this work, we find no evidence that overall RoH content or specific RoHs contribute to early-onset breast cancer risk. However, we have sequenced the exons of several genes known to be important in ER- breast cancer in order to discover new variants in these regions and assess evidence of association for less common variants using gene-level association tests.

The utility of RoH analysis for detection of cancer susceptibility loci appears to be somewhat limited, at least in case-control GWA studies using standard SNP panels measured on a few thousand individuals. The technique may be more appropriate in populations with higher RoH content. However, with available of large GWAS datasets the lack of association for RoHs can be confirmed for other cancer phenotypes.

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